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Antioxidant Activity of Two Steroid Alkaloids Extracted from *Solanum aculeastrum*

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Abstract: In this study two steroid glycosides were isolated from the berries of this plant, which were identified as tomatidine and solasodine by spectroscopic techniques. Antioxidant activities of these compounds were investigated using DPPH, ABTS and reducing power assays. The IC_{50} confirmed the antioxidant potentials of tomatidine and solasodine. DPPH free radical activity was examined at 30 and 60 min. The highest inhibition was observed when the two compounds were combined, followed by solasodine while tomatidine showed the least inhibition. On the other hand, the activity of ABTS was greater than the DPPH and the activity of the combined compounds was faintly less than solasodine. The activity observed in the reducing power assay was higher in the combined compounds and followed by solasodine and tomatidine. This study has revealed strong antioxidant activity and synergistic effect of the isolated compounds from *S. aculeastrum* berries.

Key words: *Solanum aculeastrum*, tomatidine, solasodine, antioxidant, DPPH, ABTS

INTRODUCTION

Recent developments in biomedics have pointed to the involvement of free radicals in many diseases such as cancer, atherosclerosis, diabetes, neurodegenerative disorders and aging (Yu, 1994; Halliwell and Gutteridge, 1999; Kasai *et al*., 2000; Yesilada *et al*., 2000). Although living organisms possess enzymatic and non-enzymatic defense systems against excessive production of free radicals, different external factors (smoke, diet, alcohol, some drugs) and aging, decrease the efficiency of such protecting system, resulting in the disturbance of the redox equilibrium established under healthy conditions (Ames *et al*., 1993; Zin *et al*., 2006). Antioxidants that can neutralize free radicals may therefore be used to protect the human body from diseases and retard rancidity in foods consumed by humans (Pryor, 1991; Leong and Shui, 2002). It is believed that higher intake of antioxidant rich food is associated with decreased risk of degenerative diseases particularly cardiovascular diseases and cancer (Ames *et al*., 1993; Joseph *et al*., 1999). Several research studies have demonstrated that herbal plants contain diverse classes of compounds such as polyphenols, alkaloids, tannins and carotenoids (Velioglu *et al*., 1998; Zheng and Wang, 2001). Some of these properties have been related to the action of these compounds as antioxidants, free radical scavengers, quenchers of singlet and triplet oxygen and inhibitors of peroxidation. These phytochemicals are found distributed in different parts of plants (Larson, 1988; Kim *et al*., 2002; Pilarski *et al*., 2006). There is therefore an increasing interest in finding natural herbal plants that exhibit antioxidative activity.

Solanum aculeastrum Dunal is a medicinal plant, which occurs from tropical Africa down to South Africa (Koduru *et al*., 2006c). Local healers use its extremely bitter berries for the treatment of various diseases in humans and domestic animals (Hutchings *et al*., 1996). The fresh and boiled ripe berries are used to treat jigger wounds and gonorrhoea respectively (Agnew and Agnew, 1994). Earlier discussions with traditional healers of the Eastern Cape Province in South Africa revealed that the plant is also used for the treatment of cancer (Koduru *et al*., 2006a). This claim was confirmed in our

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recent bioassay of its crude extracts on three carcinoma cell lines (Koduru *et al*., 2006c). Our previous studies have also evaluated the antimicrobial and antioxidant activities of this plant using crude extracts of its berries (Koduru *et al*., 2006a, b). In continuation of our research on the medicinal value of this plant, this study was aimed at isolating some of its active compounds and to investigate their antioxidant activity.

MATERIALS AND METHODS

Collection of Plant Berries

The berries of *S. aculeastrum* were collected from plants naturally occurring in the wild at Kayalethu village, near the town of Alice, in the Eastern Cape Province of South Africa (latitudes 30°00'-34°15'S and longitudes 22°45'-30°15'E). The plant was identified at the Department of Botany, University of Fort Hare and a voucher specimen (Vedic Med 2005/16) was prepared and deposited in the Griffen Herbarium of the University.

Extraction and Isolation of Tomatidine and Solasodine

The berries of *S. aculeastrum* were oven dried to constant weight at 60°C and ground to powder using a blender. Following the method of Weissenberg (2001), 100 g of the powder was added to 300 g toluene plus 200 mL water and 100 mL of 32% HCl and refluxed with stirring for 5 h. The reaction mixture was subsequently alkalinised with 40% aq. NaOH (200 mL) and refluxed again with stirring for 2 h. Following phase separation, the upper, pale-yellow toluene layer was siphoned off and the remaining dark brown aqueous mixture was further extracted three times with 100 mL portions of toluene. The procedure gave consistently colourless and crystalline steroid glycosides with 90-95% recovery (Weissenberg, 2001). The white precipitate was separated by preparative TLC using the solvent system $CHCl_3$-EtOAc (9:1) and the compounds were identified using NMR spectroscopy.

NMR Spectroscopy

^1H and ^{13}C NMR spectra were recorded on a Bruker AMX400 spectrometer at 400 MHZ and 100.60 MHZ, respectively. Deuterated chloroform was used as the solvent for all the NMR experiments. Chemical shifts are reported in ppm, using TMS as internal references. Preparative TLC-silica gel 60 $F_{254+366}$ was used for preparative thin layer chromatography (Merck, South Africa) and visualized using sulphuric acid spray (1% H_2SO_4 in MeOH).

DPPH Radical Scavenging Assay

The method described by Liyana-Pathiranan and Shahidi (2005) was adopted for the assessment of the DPPH radical scavenging activity of the pure compounds. (DPPH is a stable radical of purple colour which is reduced to yellow-coloured diphenylpicryhydrazine when it reacts with an antioxidant compound which can donate hydrogen. The change in colour is measured spectrophotometrically at 517 nm on a UV/Visible light spectrophotometer). A 0.135 mM DPPH solution in ethanol (1 mL) was mixed with various concentrations of each compound and vortexed thoroughly. The absorbance of the mixtures at ambient temperature was recorded at 30 and 60 min. Catechin was used as the standard antioxidant compound. The scavenging of DPPH radical was calculated according to the following equation:

DPPH radical scavenging activity (%) = $[(Abs_{control}-Abs_{sample})]/(Abs_{control})]x100$ where $Abs_{control}$ is the absorbance of DPPH radical + methanol; Abs_{sample} is the absorbance of DPPH radical + sample compound/standard.

ABTS Radical Scavenging Activity

The ABTS radical-scavenging activity was determined according to Re *et al.* (1999). This method is based on the ability of antioxidants to quench the long-lived ABTS radical cation, a blue/green chromophore with characteristic absorption at 734 nm. The ABTS radical cation was prepared by reacting an aqueous solution of ABTS (7 mM) with potassium persulfate (2.45 mM, final concentration), which was kept in the dark at 25°C for 16 h. The solution was diluted in ethanol to an absorbance of 0.70 (±0.020) at 734 nm before use. Samples were determined at 30°C, exactly 6 min after initial mixing. Appropriate solvent blanks were run in each assay. The antioxidant solution reduces the radical cation to ABTS, which reduces the color. The extent of decolorization is calculated as percentage reduction of absorbance and this is determined as a function of concentration and calculated relative to the equivalent standard (BHT) concentration. The activity of each antioxidant was determined at four concentrations, within the range of the dose response curve of standard.

Determination of the Reducing Power of the Isolated Tomatidine and Solasodine

Reducing capacity of tomatidine and solasodine was determined by the method of Oyaizu (1986). Different concentrations (1, 10, 25 and 50 μg mL^{-1}) of each compound in methanol were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide [$K_3Fe(CN)_6$]. The mixture was incubated at 50°C for 20 min. Aliquots (2.5 mL) of 10% trichloroacetic acid were added to the mixture, which was then centrifuged for 10 min at 1000 g. The upper layer of solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$ and absorbance was measured at 700 nm in a spectrophotometer.

Each experiment was replicated thrice and the data were expressed as means of measurements while IC_{50} values were calculated by extrapolation.

RESULTS AND DISCUSSION

Isolation and Identification of Tomatidine and Solasodine

The phytochemical analysis of the berries of *S. aculeastrum* afforded two steroid glycosides, tomatidine and solasodine (Fig. 1). The structures of the compounds were established with the aid of NMR spectroscopic techniques in addition to their comparison with data found in the literature (V'azquez *et al*., 1997; Weissenberg, 2001; Wanyonyi *et al*., 2002).

DPPH Free Radical Scavenging Activity of the Identified Compounds

A high radical scavenging activity was observed in tomatidine, solasodine and the mixture of both compounds (1:1) in a concentration dependent manner. Whilst the mixture of both compounds showed higher antioxidant activity between 54.6 to 64.7%, solasodine and tomatidine recorded 45.34 and 55.7 and 45.41 to 51.28%, respectively after 30 min of incubation (Fig. 2). DPPH showed slightly higher activity after 60 min incubation (Fig. 3). This activity was comparable to that of the reference antioxidant compound, catechin (Table 1). However, the IC_{50} values of the combined compounds showed higher activity than for the individual compounds, which suggested synergistic effects of the two compounds.

Table 1: IC_{50} values (DPPH and ABTS) of tomatidine and solasodine from *Solanum aculeastrum*. IC_{50} values in μg mL^{-1}

Compounds	DPPH (30 min)	DPPH (60 min)	ABTS
Catechin (control)	0.62	0.58	-
BHT (control)	-	-	0.91
Tomatidine	38.19	5.39	0.87
Solasodine	14.70	0.99	0.78
Toma:Sola (1:1)	0.92	0.90	0.80

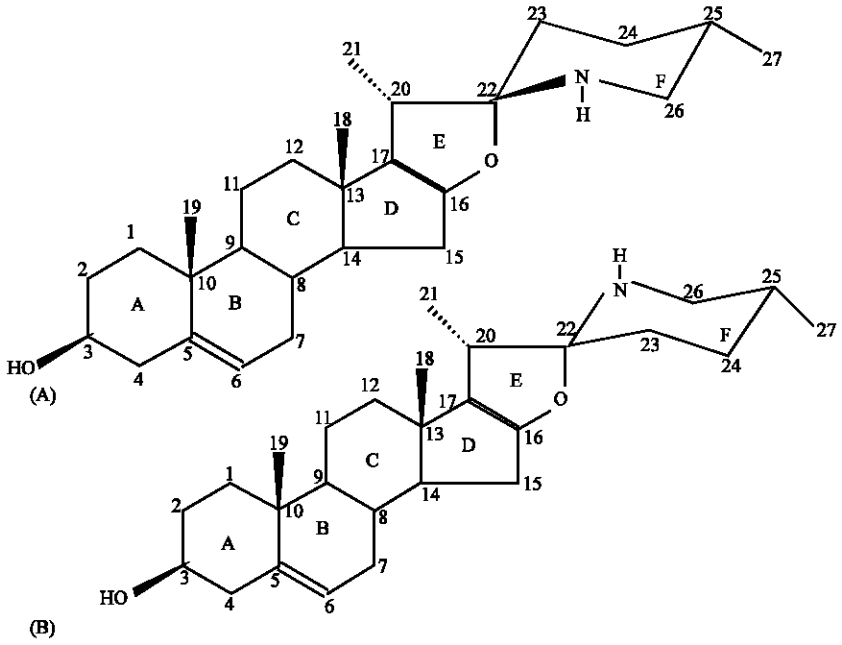

Fig. 1: The structures of tomatidine (A) and solasodine (B) isolated from the berries of *Solanum aculeastrum*

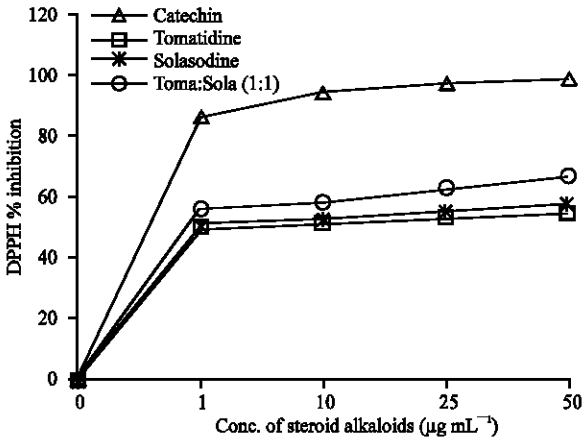

Fig. 2: DPPH radical scavenging activity of steroid alkaloids from *Solanum aculeastrum* after 30 min (Control-Catechin)

ABTS Radical Scavenging Activity of the Isolated Compounds

Tomatidine and solasodine exhibited potent scavenging activity for ABTS radical cations in a concentration dependent manner (Fig. 4), showing their direct roles in trapping free radicals. It was found that the relative ranking of the pure compounds, using the ABTS radical, was higher than the DPPH assay (Fig. 2-4). However, the activity of solasodine (85.72%) was slightly higher than that of the combined compounds (83.42%) followed by tomatidine (66.88%). The apparent lesser activity of tomatidine might be due to its insolubility in the aqueous medium.

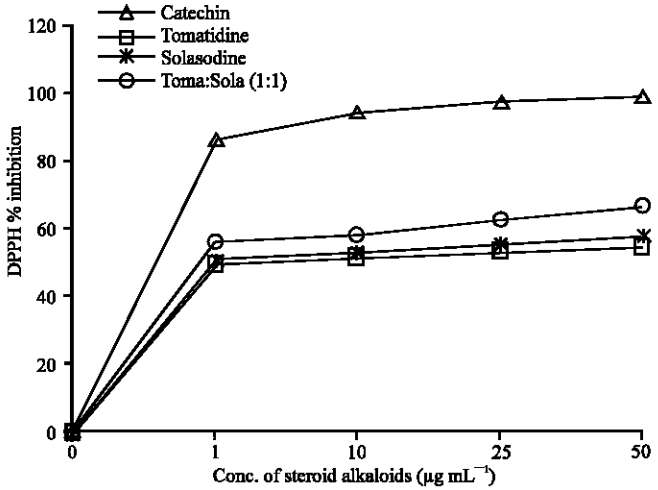

Fig. 3: DPPH radical scavenging activity of steroid alkaloids from *Solanum aculeastrum* after 60 min (Control-Catechin)

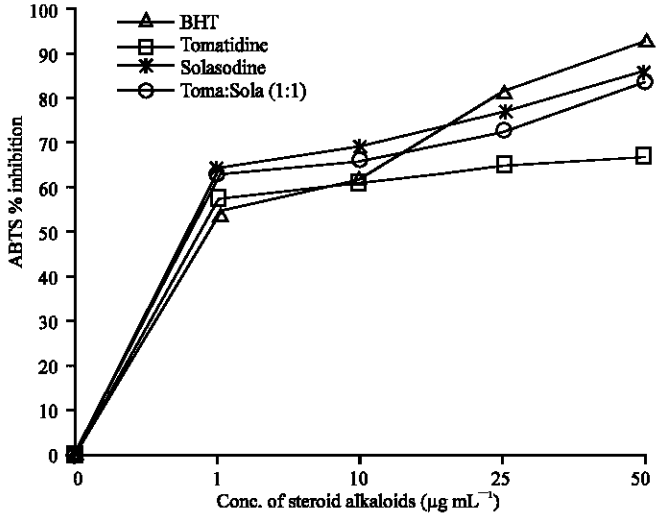

Fig. 4: ABTS radical scavenging activity of steroid alkaloids from *Solanum aculeastrum* (Control-BHT)

Determination of the Reducing Power of Tomatidine and Solasodine

The antioxidant capacity of the pure compounds was further examined by the reducing power assay. In the presence of antioxidant compounds, Fe^{3+}/ferricyanide complex is reduced to the ferrous form and the Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increased absorbance at this wavelength indicates a stronger reducing power (Behera *et al*., 2006). Figure 5 shows the dose response curves for the reducing powers of the reference compound (catechin) and the different concentrations of the isolated steroid alkaloids of *S. aculeastrum*. Like the antioxidant activity, the reducing power of the compounds increased steadily with increase in their concentration. The reducing power was in the order, combined compounds>solasodine>tomatidine. Although the reducing power of solasodine and tomatidine were significantly lower than that of catechin, when the

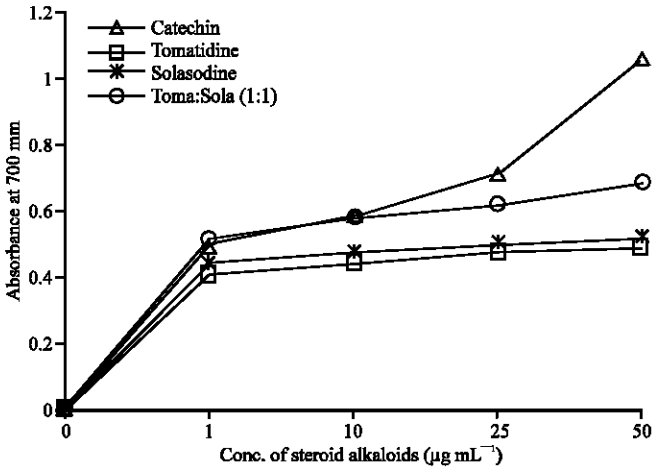

Fig. 5: Reducing power of steroid alkaloids from *Solanum aculeastrum* (Control-Catechin)

two compounds were combined, their activity was comparable to that of catechin. It therefore implies that the combined compounds could be regarded as an electron donor, capable of reacting with free radicals, thereby converting them to more stable products thus terminating the radical chain reaction (Yen and Chen, 1995). It is believed that the antioxidant activity of a substance and its reducing power are related (Duh, 1998; Duh *et al*., 1999; Tanaka *et al*., 1988).

S. aculeastrum grows widely in South Africa and is used for the treatment of many infectious illnesses (Koduru *et al*., 2006a). In recent years, it has been used clinically to treat cancers and has demonstrated the ability to inhibit tumour development (Koduru *et al*., 2006c). In our previous investigation, its crude extracts have shown significant antioxidant activity (Koduru *et al*., 2006b). The present study has shown that tomatidine and solasodine may be responsible for the antioxidant activity of the crude extracts. This study also showed for the first time that the two alkaloids, tomatidine and solasodine, have synergistic effect.

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